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Ceftriaxone bone penetration in patients with septic non-union of the tibia

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SUMMARY

Objectives: A main determinant of clinical response to antibiotic treatment is drug concentration at the infected site. Data on ceftriaxone (CFX) bone penetration are lacking. We measured CFX concentrations in infected bone to verify their relationship with pharmacodynamic microbiological markers.
Methods: Eleven patients undergoing debridement for septic non-union of the tibia and receiving intravenous CFX were studied. Plasma and bone specimens were collected intraoperatively at a variable interval after CFX administration. Drug concentrations were measured by high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method.
Results: Bone samples were extracted at a mean of 3.3 h (range 1.5–8.0 h) since the start of CFX infusion. The mean ± standard deviation intraoperative CFX plasma concentration was 128.4 ± 30.8 mg/l; the corresponding bone concentrations were 9.6 ± 3.4 mg/l (7.8%) in the cortical compartment and 30.8 ± 8.6 mg/l (24.3%) in the cancellous compartment. The mean 24-h area under the concentration–time curve (AUC_{24}) values were 176.8 ± 62.2 h*mg/l in cortical bone and 461.5 ± 106.8 h*mg/l in cancellous bone. The time above the minimum inhibitory concentration (T>MIC) was 24 h in all compartments. The estimated mean free AUC/MIC ratios and T>MIC were 140 and 24.4 h, respectively, in cancellous bone and 42.4 and 21 h, respectively, in cortical bone.
Conclusions: CFX bone penetration was poor (<15%) in the cortical compartment and satisfactory in the more vascularized cancellous bone. The T>MIC and AUC/MIC ratios suggest that CFX achieves a satisfactory pharmacokinetic exposure in cancellous bone as far as pathogens with a MIC of <0.5 are concerned. However, considering free drug concentrations, pharmacokinetic/pharmacodynamic targets may not be fully achieved in cortical bone. As antibiotic exposure can be suboptimal in the infected cortical compartment, and drug penetration may be impaired into necrotic bone and sequesters, a radical surgical removal of purulent and necrotic tissues appears essential to shorten treatment duration and to prevent treatment failures.

1. Introduction

In spite of medical progress, the management of bone and joint infections remains problematic, requiring prolonged courses of parenteral antibiotics and surgical debridement in most cases. Bone infections tend to involve sites of relatively poor vascularity and are mainly caused by Gram-positive organisms, especially staphylococci, although Gram-negative agents may also play a relevant role.[1–3]

Given their broad spectrum of activity, third-generation cephalosporins are commonly used to treat osteoarticular infections either as a single regimen or in association with an anti-staphylococcal agent. Intravenous antimicrobial treatment of osteomyelitis increasingly takes place in outpatient settings; in this context, ceftriaxone (CFX) as once-daily treatment is a valuable option, given its unique pharmacokinetics, with prolonged plasma half-life, and broad spectrum of activity, including against community-acquired bacteria involved in post-traumatic and/or hematogenous osteomyelitis.[4]

A major determinant of the clinical response to antimicrobial treatment is the drug concentration at the infected site.[5,6] In infected bone, activation of the inflammatory process may result initially in increased vascular permeability, with local edema, followed by impaired blood supply, necrosis and sequester formation; in the latter situation, antimicrobial penetration may be inefficient and surgery may become essential. The study of antimicrobial penetration into infected bone after multiple dosing better estimates the in vivo situation under infection and may

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provide useful information for optimizing the dosage and the choice of antimicrobials. Data on CFX penetration into human bone are limited and most come from studies on prophylaxis rather than on therapy, as antibiotic penetration into non-infected human bone has been evaluated on samples collected during knee or hip arthroplasty after a single preoperative dose of antimicrobial. Furthermore, differences in terms of general methodology, drug extraction, and measurement make these studies difficult to compare.

We undertook a clinical pharmacokinetic study on patients with septic pseudoarthrosis receiving CFX as a part of the antimicrobial treatment, in order to measure bone CFX penetration under steady-state conditions and verify the relationship between tissue concentrations and the minimum inhibitory concentration (MIC) of the infecting agents.

2. Materials and methods

2.1. Patients

Adult orthopedic patients undergoing surgical debridement for septic non-union of the tibia with resection of infected and necrotic bone and treated for more than 1 week with intravenous CFX were studied. A patient was included in the present study when all of the following criteria were met: (1) The patient should have clinical and radiological evidence of septic pseudoarthrosis, defined as the presence of inflammation or fistula in the area of a previous bone fracture, radiological non-union of the bone involved, and/or the presence of biological inflammatory syndrome. Biological inflammatory syndrome included an erythrocyte sedimentation rate (ESR) of >50 mm/h and elevated levels (>10 mg/dl) of C-reactive protein (CRP). (2) Indication for surgical debridement of infected/necrotic bone and external fixation treatment. (3) The patient should be on current intravenous antibiotic treatment with CFX at the time of surgical intervention, of at least 7 days duration, to guarantee the steady-state attainment; antibiotic treatment should respond to "best practice and standard of care guidelines" as to indication and dosage.[7,8] Exclusion criteria were moderate to severe renal or hepatic impairment, intolerance or contraindication to the use of cephalosporins, and infection sustained by CFX-resistant pathogens.

At the time of inclusion, demographic, clinical, and radiological data were registered, including co-morbidities, concomitant treatments, weight and height. Laboratory data, collected both prior to and after surgery, included blood and differential counts, kidney and liver function tests, ESR, CRP, total proteins and albumin levels. The study period started in January 2006 and ended in January 2009. The study was performed according to the current revised version of the Declaration of Helsinki, and written informed consent was obtained from each patient. The study was a non-interventional one. The paper was reviewed and approved by the local institutional review board.

2.2. Sample collection

Bone specimens were collected during surgical debridement of necrotic and infected tissue, at a variable interval from the start of antibiotic infusion, depending upon surgical timing. Simultaneously with bone resection, peripheral venous blood samples were collected using 7-ml lithium–heparin BD Vacutainer system vials. The amount of tissue vascularization and the eventual presence of avascular bone were determined by histopathological analysis of a fragment of collected bone; on the basis of the results, necrotic tissues and sequesters were excluded from evaluation. Another bone sample was cultured for microbiological assays and etiological determination. Clinical specimens were processed using standard microbiological procedures. Species identification of the isolates was performed using the API 20E gallery (BioMérieux, Marcy l'Etoile, France), routinely used in our laboratory. Antimicrobial susceptibility tests of all isolates was confirmed with the disk diffusion method, and results were evaluated according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[9]

Serum was obtained by blood centrifugation at 3000 rpm for 10 min and was stored at −20 °C prior to assay. Bone samples used for pharmacokinetic analysis were dissected into cortical and cancellous bone, washed for 10 s with 10 ml of sterile saline to remove blood and coagules, blotted dry and stored at −80 °C. Bone marrow was extracted and was not analyzed.

At the time of analysis, cortical specimens were defrosted, cleaned of soft tissue, and crushed into powder with an analytic mill (IKA A11 basic; IKAWerke, GmbH, Staufen, Germany). Cancellous bone samples were thawed and ground up using a porcelain pestle and mortar. Crushing procedures consisted of 15-min grinding cycles, at intervals of 10 s each, to avoid temperature increases above the thermal stability threshold of CFX. For both cortical and cancellous bone, several samples of 500 mg were weighed with a precision electronic balance and transferred into 2.5-ml plastic vials. To quantify CFX in plasma and bone, a fully validated high-performance liquid chromatography (HPLC) assay was used.

2.3. Stock solutions, plasma standards, and quality controls

CFX stock solutions were prepared in water at a concentration of 1 mg/ml. Standard samples were prepared by serial dilutions of the highest standard prepared after addition of determined volumes of stock solutions to blank plasma. Calibration curves ranged from 250 mg/l to 0.97 mg/l. To improve the accuracy and precision of the method, three levels of quality control (high, medium and low) were prepared by successive dilutions of stock solutions with blank plasma.

2.4. Bone standards and quality controls

Similarly to plasma, the unknown bone sample concentration was calculated from a linear calibration curve. To obtain standard bone samples, the stock solution of each drug was diluted in blank weighed bone. Calibration curves of five points, including blank bone, were obtained from standard samples and ranged from 25 μg/g to 3 μg/g. Three levels of bone quality control were prepared: high, medium and low.

2.5. HPLC equipment

The chromatography apparatus was a Merck–Hitachi LaChrom (Tokyo, Japan), with a pump model L-7100, an L-7200 autosampler, an L-7400 ultraviolet detector, and D-7000 interface. HPLC System Manager software (HSM version 4.1; Merck–Hitachi, Tokyo, Japan) was used for managing the HPLC system. Chromatographic separation was performed by Atlantis 3 μ C18 column (150 × 4.6 mm i.d.; Waters SpA, Milan, Italy) protected by C18 Security-Guard (4.0 × 3.0 mm i.d.; Phenomenex, CA, USA) at 35 °C, using a column thermostat L-7350 Merck–Hitachi LaChrom. Assay separation was achieved by gradient elution and the mobile phase was composed of buffer A (KH_2PO_4 50 mM) and acetonitrile as buffer B. The flow rate was set at 1 ml/min. The UV detector was set at 274 nm, which is the best wavelength in terms of selectivity and sensibility for CFX analysis.

2.6. Ceftriaxone assay

Plasma samples were prepared by mixing 200 μl of plasma with 600 μl of acetonitrile and 50 μl of internal standard (IS)

(50 mg/l of thymidine (from Sigma-Aldrich), in water, prepared daily). After vortexing for at least 10 s and centrifugation at 12 000 rpm for 10 min at 4 °C, 400 μl of supernatant was diluted with 400 μl of water, and 50 μl of sample was injected into the HPLC system. Bone samples were mixed with 50 μl of IS and with 1 ml of sodium phosphate buffer pH 7 (Sigma-Aldrich, Italy). Samples were vortexed for 10 s and tumbled for 5 h at 25 rpm at 4 °C. After centrifugation at 12 000 rpm for 10 min at 4 °C, 20 μl of supernatant was injected into the HPLC apparatus. The calculation method was based on peak areas and on internal standard ratio; the type of calibration curve selected was "linear and forced through zero". The retention time of CFX was 8.2 (± 0.2) min and for IS was 3.8 (± 0.2) min. Accuracy, intra- and inter-day variability expressed as coefficient of variation % (CV%) were respectively 5.2%, 3.6% and 4.5% for plasma samples, and 7.2%, 7.8% and 10.3% for bone samples. Recovery was 86% (CV% = 3) for bone samples and 82% (CV% = 8) for plasma samples. Detection limits for the CFX assay in plasma and bone were 0.24 mg/l and 0.5 mg/l, respectively.

The amount of antibiotic recovered in bone samples due to blood contamination was calculated as previously described,[10,11] with the following formula:

$$\text{Blood contamination}(\%) = K\frac{\text{Hb in supernatant}}{\text{Hb in blood}}(100 - \text{Hct}),$$

where Hb is hemoglobin (g/dl), K the dilution factor corresponding to the mean volume of water displaced by 1 g of bone (1/bone density) and measuring 0.83 ml for cortical bone and 2 ml for cancellous bone,[10] and Hct is the hematocrit (%). The net bone concentration was obtained by subtracting blood contamination from the value measured in bone samples.

Antibiotic concentrations were measured in duplicate in adjacent bone samples and then averaged to improve the accuracy of the assays.

2.7. Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis of the data was performed using the WinNonlin software program (WinNonlin version 5.2; Pharsight Corp., Mountain View, CA, USA). For each patient, drug concentrations measured in plasma and bone were plotted against time of sampling. The first-order approximation was used to derive 'population pharmacokinetic' parameters, such as CFX half-life; the maximum concentrations (C_{max}) and minimum concentrations (C_{min}) in plasma and bone were subsequently obtained from the known data. The 24-h area under the concentration–time curve (AUC_{24}) in plasma and bone was calculated by linear-log trapezoidal rule.

2.8. Statistical analysis

Statistical analysis was performed using PASW Statistics version 17.0 (IBM SPSS, Chicago, IL, USA). Statistical decisions were made at $p = 0.05$. All p-values were two-tailed. To evaluate linear relationships, Pearson and Spearman correlation tests were used.

3. Results

Eleven male patients were studied; their mean age was 37.8 years (range 18–65 years). Demographic characteristics of the study population are shown in Table 1.

All patients received intravenous CFX 2 g daily administered over a 30-min infusion for post-traumatic septic non-union of the diaphyseal tibia and underwent surgical debridement of infected and necrotic bone. Mean duration of treatment before sampling was 15.8 days. Bone cultures grew methicillin-sensitive *Staphylococcus aureus* (MSSA) in four cases, *Staphylococcus epidermidis* (MSSE) and *Serratia marcescens* in two cases each, and *Citrobacter freundii*, *Klebsiella oxytoca* and *Escherichia coli* in the remaining patients. All isolates were sensitive to CFX (MIC <2 mg/l).

Bone samples were extracted at a mean of 3.3 h (range 1.5–8.0 h) since the start of CFX infusion. Median and mean ± standard deviation (SD) CFX plasma concentrations at the time of osteotomy were 118.5 and 128.4 ± 30.8 mg/l, respectively. Median and mean ± SD bone concentrations were 9.1 and 9.6 ± 3.4 mg/l, respectively, in cortical bone and 28.4 and 30.8 ± 8.6 mg/l, respectively, in cancellous bone. The mean bone/plasma concentration ratio was 7.8% for cortical bone and 24.3% for cancellous bone (Table 2). The distribution of plasma and bone CFX concentrations and of bone/plasma ratios over time are illustrated in Figures 1 and 2.

The mean peak concentration in plasma (C_{max}), derived by a population pharmacokinetic analysis, was 191.6 mg/l; the mean plasma volume of distribution at steady state (V_{ss}) was 9.06 l. The plasma half-life was 5.9 h; the lambda z was 0.1175 l^{-1}. The median and mean ± SD plasma AUC_{24} were 1902.1 and 1930.5 ± 310.8 h*mg/l, respectively. The median and mean ± SD bone AUC_{24} were 163.2 and 176.8 ± 62.2 h*mg/l, respectively, for cortical bone, and 459.4 and 461.5 ± 106.8 h*mg/l, respectively, for cancellous bone. Bone half-life was 16.6 h in cortical and 7.3 h in cancellous bone.

The median and mean ± SD values for CFX AUC_{24} bone/plasma ratio were 9.1% and 9.3 ± 3.2%, respectively, for cortical bone and 24.2% and 24.1 ± 5.1%, respectively, for cancellous bone (Table 3). The mean overall drug exposure in cancellous bone was three times higher than in cortical bone. MICs of infecting bacteria ranged between 0.25 and 0.5 mg/l. The mean CFX concentration over the MIC

Table 1
Demographic characteristics of the study population

Patient number	Age (years)	Weight (kg)	Height (m)	BMI	Days of treatment[a]	Co-morbidity	Co-medication
1	18	46	1.62	17.53	9	Crohn's disease	Analgesics, LMW heparin, prednisone
2	41	75	1.80	23.15	17	Asthma	Analgesics, LMW heparin, salmeterol, fluticasone
3	30	65	1.65	23.88	11	-	Analgesics, LMW heparin
4	24	83	1.82	25.06	10	-	Analgesics, LMW heparin
5	38	73	1.76	23.57	18	HCV infection	Analgesics, LMW heparin
6	36	81	1.78	25.56	8	-	Analgesics, LMW heparin
7	38	79	1.72	26.70	12	-	Analgesics, LMW heparin
8	33	77	1.80	23.77	32	-	Analgesics, LMW heparin
9	29	73	1.74	24.11	30	-	Analgesics, LMW heparin
10	65	80	1.65	29.38	15	Dyslipidemia	Analgesics, LMW heparin pravastatin
11	64	70	1.60	27.34	12	Hypertension	Analgesics, LMW heparin, olmesartan/hydrochlorothiazide
Mean	37.8	72.9	1.72	24.55	15.8		

BMI, body mass index; LMW, low molecular weight; HCV, hepatitis C virus.
[a] Duration of antibiotic treatment with ceftriaxone before sampling.

Table 2
Ceftriaxone concentrations in plasma and cortical and cancellous bone

Patient number	Time of sampling (min)[a]	Plasma concentration (mg/l)	Cortical bone concentration (mg/l)	Cancellous bone concentration (mg/l)	Cortical bone/ plasma ratio (%)	Cancellous bone/ plasma ratio (%)	Blood contamination cortical bone (%)	Blood contamination cancellous bone (%)
1	90	195.97	6.95	35.65	3.54	18.19	0.67	3.21
2	120	166.11	17.28	36.84	10.40	22.18	1.05	8.89
3	135	142.16	6.08	38.08	4.27	26.78	0.82	3.96
4	150	133.85	5.68	49.42	4.24	36.92	1.75	8.44
5	150	129.12	9.72	31.11	7.53	24.09	1.34	9.89
6	165	110.02	13.48	27.57	12.25	25.06	1.34	9.89
7	180	109.93	11.64	28.43	10.59	25.86	1.46	10.55
8	210	118.34	9.44	22.83	7.97	19.29	1.54	4.21
9	225	118.50	9.10	20.58	7.68	17.36	1.43	4.30
10	270	98.80	7.54	25.17	7.63	25.47	1.55	2.49
11	480	89.52	8.83	22.99	9.86	25.69	0.22	5.34
Mean	197.7	128.39	9.61	30.79	7.82	24.26	1.17	6.09
Median	165	118.5	9.10	28.43	7.68	25.06	1.34	5.34

[a] Time past start of infusion (duration of infusion = 30 min).

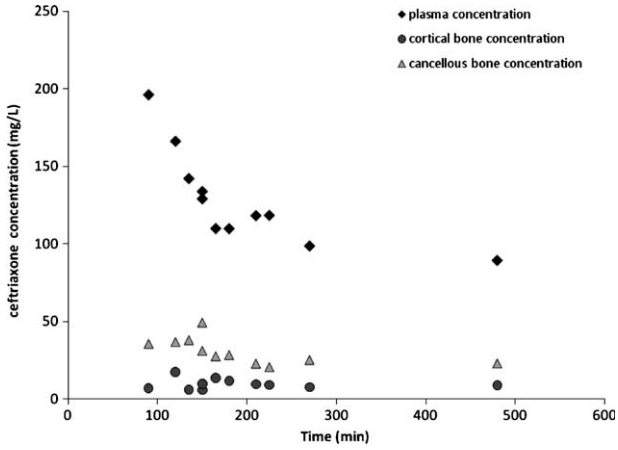

Figure 1. Ceftriaxone concentrations in plasma and bone vs. time.

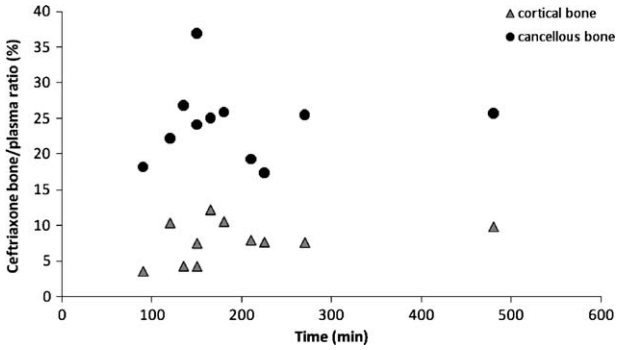

Figure 2. Ceftriaxone bone/plasma ratios vs. time.

was 21.7 for cortical bone and 73.1 for cancellous bone (Table 4). Mean CFX AUC_{24}/MIC ratios were 4516.7 for plasma, 400.1 for cortical bone, and 1092.6 for cancellous bone. CFX concentrations were higher than the MIC of the infecting agent for the entire dosing interval in all compartments evaluated.

Assuming unbound CFX to be about 10% of the total, the estimated mean free AUC was 56.1 h*mg/l in cancellous bone and 17.9 h*mg/l in cortical bone; the corresponding mean free AUC/MIC ratios and T>MIC were 140 and 24.4 h, respectively, in cancellous bone and 42.4 and 21 h, respectively, in cortical bone.

Nine patients were clinically and radiologically cured at the latest follow-up visit. One patient (patient 11) underwent a leg amputation and one (patient 7) had chronic osteomyelitis.

3.1. Statistical analysis

Statistically significant results of the correlation analysis are listed in Table 5.

Pearson's correlation coefficient indicated a statistically significant linear relationship between CFX cortical bone concentrations

Table 3
Ceftriaxone pharmacokinetic parameters in plasma and cortical and cancellous bone

Patient number	Plasma V_{ss} (l)	Plasma AUC_{24} (h*mg/l)	Cortical bone AUC_{24} (h*mg/l)	Cancellous bone AUC_{24} (h*mg/l)	Cortical AUC/ plasma AUC (%)	Cancellous AUC/ plasma AUC (%)
1	8.39	2628.23	128.86	536.24	4.90	20.40
2	10.55	2290.79	331.96	496.76	14.49	21.69
3	7.48	1989.71	118.74	528.78	5.97	26.58
4	9.46	1902.06	112.93	697.10	5.94	36.65
5	9.23	1956.32	178.21	459.43	9.11	23.48
6	7.91	1614.04	214.40	405.36	13.28	25.11
7	7.43	1613.10	213.14	406.84	13.21	25.22
8	11.20	1796.33	163.19	326.33	9.08	18.17
9	10.53	1799.83	162.02	325.13	9.00	18.06
10	8.95	1616.56	137.04	404.07	8.48	25.00
11	8.56	2028.80	184.51	490.22	9.09	24.16
Mean	9.06	1930.53	176.82	461.48	9.32	24.05
Median	8.95	1902.06	163.19	459.43	9.08	24.16

V_{ss}, mean plasma volume of distribution at steady-state; AUC_{24}, 24-h area under the concentration–time curve; AUC, area under the curve.

Table 4
Ceftriaxone bone concentration/MIC ratios for Gram-positive and Gram-negative isolates

Patient number	Pathogen	Ceftriaxone MIC	Cortical bone concentration/ MIC	Cancellous bone concentration/ MIC	Plasma AUC/MIC	Cortical bone AUC/MIC	Cancellous bone AUC/ MIC	Cortical bone T>MIC (h)	Cancellous bone T>MIC (h)
1	*E. coli*	0.5	13.89	71.31	5256.47	257.72	1072.47	24	24
2	MSSA	0.5	34.56	73.67	4581.58	663.92	993.53	24	24
3	MSSA	0.25	24.30	152.30	7958.84	474.97	2115.13	24	24
4	MSSA	0.5	11.36	98.83	3804.13	225.85	1394.19	24	24
5	*C. freundii*	0.5	19.44	62.22	3912.64	356.42	918.86	24	24
6	MSSE	0.5	26.95	55.14	3228.08	428.79	810.71	24	24
7	*S. marcescens*	0.5	23.28	56.86	3226.21	426.28	813.67	24	24
8	*S. marcescens*	0.5	18.87	45.67	3592.67	326.37	652.67	24	24
9	MSSE	0.5	18.20	41.15	3599.67	324.04	650.27	24	24
10	MSSA	0.25	30.17	100.66	6466.24	548.17	1616.29	24	24
11	*K. oxytoca*	0.5	17.66	45.99	4057.60	369.03	980.43	24	24
Mean			21.7	73.07	4516.74	400.14	1092.57	24	24

MIC, minimum inhibitory concentration; AUC, area under the curve; T>MIC, time above the MIC; MSSA, methicillin-sensitive *Staphylococcus aureus*; MSSE, methicillin-sensitive *Staphylococcus epidermidis*.

Table 5
Statistically significant correlations

Variable	Associated variables	*r*	*p*-Value
Plasma concentration	Weight	−0.692	0.027
	BMI	−0.907	0.000
	Plasma AUC_{24}	0.849	0.002
Cortical bone concentration	WBC	0.924	0.000
	ESR	0.694	0.026
	Cortical bone/plasma ratio	0.807	0.005
	Cortical bone AUC_{24}	0.973	0.000
Cancellous bone concentration	Cancellous bone/plasma ratio	0.648	0.043
	Cancellous bone AUC_{24}	0.909	0.000
Cortical bone/plasma ratio	WBC	0.737	0.015
	Cortical bone concentration	0.807	0.005
	Cortical bone AUC_{24}	0.747	0.013
Cancellous bone/plasma ratio	Cancellous bone concentration	0.648	0.043
	Cancellous bone AUC_{24}	0.713	0.021
Plasma AUC_{24}	Plasma concentration	0.849	0.002
	Weight	−0.823	0.003
	BMI	−0.814	0.004
Cortical bone AUC_{24}	WBC	0.932	0.000
	ESR	0.777	0.008
	CRP	0.694	0.026
	Cortical bone concentration	0.973	0.000
	Cortical bone/plasma ratio	0.747	0.013
Cancellous bone AUC_{24}	Cancellous bone concentration	0.909	0.000
	Cancellous bone/plasma ratio	0.713	0.021

BMI, body mass index; AUC_{24}, 24-h area under the concentration–time curve; WBC, white blood cell count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.

and white blood cell count (WBC) and ESR values, between cortical bone AUC and all inflammatory indices, and finally between cortical bone penetration and WBC. Plasma CFX concentrations and plasma AUC were inversely related to body weight and body mass index (BMI).

Both in the univariate and multivariate analysis, CFX cancellous bone concentrations and cancellous bone AUC were directly related to time of collection, ESR, and CRP.

4. Discussion

CFX is frequently used in the treatment of osteoarticular infections, given its broad spectrum of activity, unique pharmacokinetics, and good tolerability. Major aspects of CFX pharmacokinetics include a high serum protein binding and a long half-life of 6 to 9 h; the latter allows a once-daily dosing regimen, which is greatly responsible for CFX cost-effectiveness when compared to other third- and fourth-generation cephalosporins. CFX binds tightly to albumin with a serum binding ratio far higher than that observed for most third- and fourth-generation cephalosporins (nearly 95% versus 10–40%); however, CFX protein binding is reversible and saturable, meaning that the binding decreases with increasing plasma concentrations.[12,13] CFX is active against community-acquired Gram-positive bacteria, but also against numerous Gram-negative agents that may be involved in post-traumatic and/or hematogenous osteomyelitis.

Although drug penetration in infected tissues is critical for efficacy, the knowledge on pharmacokinetics of antimicrobials in the bone compartment is rather sparse. To the best of our knowledge, there are three studies concerning bone penetration of CFX administered for surgical prophylaxis. Scaglione et al. measured CFX concentrations in uninfected bone of patients undergoing hip arthroplasty: CFX concentrations in cancellous and cortical bone were dramatically lower than total serum levels, but comparable to serum free levels; maximum observed CFX concentrations were 10.7 and 19.4 mg/kg of body weight in cortical and cancellous bone, respectively, 2.5 h after antibiotic administration.[14] Lovering et al. measured bone concentrations of CFX and cefamandole administered simultaneously before total hip replacement: CFX and cefamandole mean bone penetration appeared similar (15.6% vs. 18.4%), providing no evidence that cephalosporins with lower serum protein binding penetrate bone better than ones with higher protein binding.[15] Finally, Soudry et al. reported CFX penetration rates varying between 5% and 8% in

cortical bone and between 14% and 21% in cancellous bone.[16] No studies are available concerning CFX penetration into infected bone. In the present study, 11 patients with rather comparable forms of septic non-union of the tibia were investigated. At the time of pharmacokinetic sampling all patients were receiving CFX and were pharmacologically classified as being at steady-state. We analyzed CFX concentrations reached in both cortical and cancellous infected bone using a standard validated HPLC method. Necrotic bone samples and sequesters were not included in the analysis, considering that tissue drug delivery may be affected by impaired blood supply (e.g., peripheral ischemia). The mean CFX bone/plasma rate varied according to the compartment considered: cancellous bone/plasma concentration and AUC ratios were roughly three times higher than the cortical ones. The anatomic difference in vascularization between the two bone compartments may partly explain the difference observed: cancellous bone has a rich capillary bed and a relatively small volume of interstitial fluid, and drugs readily diffuse across the capillary surface area; conversely, the penetration of antibiotics into cortical bone occurs exclusively through the bone marrow or periosteal circulation and is expected to be lower than in cancellous bone.

A due consideration in the analysis of drug bone penetration is that single point measurement of bone/plasma ratios may yield significantly different results depending upon the time of sampling: after intravenous administration of an antimicrobial, tissue distribution occurs, but the shape of the concentration vs. time curves of plasma and tissue compartments may differ substantially due to delayed equilibrium between the two compartments.[17] To better estimate bone drug exposure of CFX, we modeled our data using an extrapolation approach: from plasma and bone concentrations measured at different intervals from drug administration we inferred the AUC_{0-24} values for plasma and for the two bone compartments, and we estimated bone penetration according to the respective AUC ratios.

Analyzing our results according to the classification proposed by Boselli et al.,[18] CFX can be placed among the antibiotics with satisfactory bone penetration (rates between 15% and 30%) when considering cancellous bone, and within the group with poor bone penetration (<15%) when considering cortical bone. The review published in 1999 by Boselli et al. attributed a medium bone penetration to CFX, as to second- and third-generation cephalosporins in general; however, heterogeneous studies were analyzed and cortical and cancellous bone were seldom differentiated.

In our study, inter-individual variability observed in cortical and cancellous bone concentrations may be accounted for by the different intensity of the inflammatory process in bone samples and the different timing of bone collection. Bone concentrations were found to have no association with individual variables like age, body weight, height and BMI, while an association was found in some cases between bone concentrations and inflammatory markers, perhaps as a result of increased vascularization and vascular permeability under inflammation conditions.

Tissue penetration is an estimate of the capacity of an antimicrobial to reach the site of infection. However, in the choice of the optimal antimicrobial treatment, other factors should be considered. The bacterial sensitivity to antimicrobials is expressed by parameters such as the MIC and the inhibitory quotient (IQ), the latter being the ratio between concentration and MIC. The IQ provides indications on antibiotic activity at a precise site of infection against a certain microorganism and thus on the probability of therapeutic success; therefore, the IQ is a rough estimate of the antimicrobial efficacy, but its value may vary according to the time at which antimicrobial concentration is measured.[19]The percentage of time above the MIC (%T>MIC) is regarded as the best pharmacokinetic/pharmacodynamic (PK/PD) parameter for predicting the clinical efficacy of β-lactams. CFX displays a time-dependent killing, and time to bacterial eradication has been demonstrated to correlate with the time above the MIC (T>MIC) and the area under the inhibitory time curve (AUIC, or AUC/MIC). The pharmacodynamic properties of CFX have been investigated in different populations, including healthy volunteers, children, the elderly, and patients with renal and hepatic impairment, against various Gram-positive bacteria. Target free AUIC values of at least 125 have been suggested for patients with severe infections.[20–23]

It is very likely that the same PK/PD indices also apply to bone infections, even if PK/PD targets have not yet been identified. In our study the %T>MIC corresponded to 100% of the dosing interval for total drug concentrations. Assuming unbound CFX to be about 10% of the total,[13] estimated mean free AUC/MIC ratios and T>MIC were respectively 140 and 24.4 h in cancellous bone and 42.4 and 21 h in cortical bone, suggesting that CFX achieves a satisfactory exposure in cancellous bone, as far as susceptible pathogens are considered. In contrast, CFX PK/PD targets may not be fully achieved in cortical bone, where exposure may be suboptimal.

The majority of our patients were clinically and radiologically cured at the latest follow-up visit. The recovery of bacteria in bone samples after more than 8 days of CFX treatment may be accounted for by the metabolically quiescent status and low replication rate that often characterize bone-infecting organisms; however, this underlines the importance of a proper and early surgical removal of infected bone. Indeed, treatment of osteomyelitis typically requires prolonged courses of antibiotics associated with a radical surgical debridement. The correlation between CFX bone concentration and microbiological outcome is yet to be established.

Our study has several main limitations, the first of which is the lack of a control group of non-infected human bone. The second is the different post-dose timing in bone collection, which was determined by individual surgical reasons, and the availability of a single measurement from each compartment. Third, although all samples were extracted from the same bone segment (tibia), differences in the local degree of vascularization and inflammation can account for some inter-individual variability in CFX bone concentrations. Finally, the method of drug extraction from bone samples has intrinsic limitations and does not differentiate intra- and extra-cellular compartments; incomplete extraction and consequent underestimation cannot be excluded. Drug removal during sample washing was minimized by the short duration of the procedure; drug levels in the washing fluid were in all cases under the detectability level. Drug degradation may occur during grinding without cooling; we thus performed short-term crushing cycles to avoid temperatures rising up to the CFX thermal instability threshold. The size of the study population appears to be clinically relevant in consideration of the small case-series so far reported in the literature.

In conclusion, the analysis of the degree of penetration of antimicrobials into infected tissues aims at giving clinicians additional information on which to base the choice of the optimal antibiotic treatment. In our study, CFX displayed a poor penetration into septic cortical bone, while bone penetration was satisfactory into the highly vascularized cancellous bone. As antibiotic exposure can be suboptimal in the infected cortical compartment, and drug penetration may be impaired into necrotic bone and sequesters, a radical surgical removal of purulent and necrotic tissues appears essential to shorten treatment duration and to prevent treatment failures.

Conflict of interest: No conflict of interest to declare.

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